# MECHANISM OF ACTION OF DL-19-NOR-D-HOMOTESTOSTERONE ON INTERFERON FORMATION IN CELL CULTURES

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The compound DL-19-nor-D-homotestosterone differed in its effects on interferon synthesis in chick embryonic cell cultures induced by influenza virus and on the synthesis of total cell protein: the inhibition of interferon synthesis was irreversible, while inhibition of synthesis of total cell protein was reversible. The phase of interferonogenesis sensitivity to the action of the hormone lasted not less than 5 and not more than 10 h after infection with the interferonogen.

The writers have previously shown that the synthetic testosterone analog DL-19-nor-D-homotestosterone (DLNDH) inhibits interferon production in chick embryonic cell cultures without affecting exogenous interferon activity [1]. This inhibition of interferonogenesis was accompanied by inhibition of cell protein synthesis by 50%. It was concluded from these results that large doses of DLNDH exert a parallel action on interferon synthesis and on total cell protein synthesis. However, in the course of the investigations certain differences between the action of DLNDH on these two processes came to light.

The results of a further study of the effect of DLNDH on interferon synthesis are described below.

## EXPERIMENTAL METHOD

Preparation of the culture of chick embryonic tissues, the preparation of interferon induced by infecting this culture with influenza virus B, strain Lee, and the methods of interferon titration and of estimation of the level of total cell protein synthesis were described in the previous paper [1]. Protein was determined by the method of Lowry and co-workers [4]. In all experiments, DLNDH was used in a concentration of 50 μg/ml.

## EXPERIMENTAL RESULTS

The influence of experimental conditions on manifestation of the inhibitory effect was first studied. Inhibition of total cell protein synthesis required the presence of DLNDH throughout the experiment (Table 1), and removal of the hormone 3 h before introduction of the isotope abolished the inhibitory effect.

Different results were obtained when the effect of conditions of incubation of the hormone with the cells on its ability to inhibit interferon production was studied. The conditions of these experiments were similar to those of the experiments to study the effect of DLNCH on protein synthesis: the hormone was added 20 h before infection of the cell culture with the inducing virus and was removed by washing the cells thoroughly after different periods of time. The results given in Table 1 show that marked inhibition of interferon synthesis also took place if DLNDH was present throughout the experiment, but washing the cells to remove hormone 3 h before addition of the interferonogen or simultaneously with it did not change the

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TABLE 1. Reversibility of Action of DL-19-nor-D-homotestosterone (DLNDH) on Total Cell Protein Synthesis and Interferon Synthesis

| Time of removal of hormone before introduction of isotope or interferonogen (in h) | Effect on synthesis of cell protein | | Effect on interferonogenesis | |
|---|---|---|---|---|
| | incorporation of glycine-$C^{14}$ (in pulses/min/mg protein) | inhibition (in percent) | titer of interferon (in $PDD_{50}$) | inhibition of interferon production (in %) |
| 3 | 2,990 | 0 | 54 | 92 |
| 0 | not investigated | | 12 | 98 |
| – | 1,425 | 52 | 47 | 93 |
| Control (without hormone) | 2,973 | 0 | 660 | 0 |

Note: – means that the hormone was not removed.

TABLE 2. Determination of Sensitivity of Various Phases of Interferonogenesis to Action of DLNDH

| Time of addition of hormone before or after introduction of virus (in h) | Titer of interferon (in $PDD_{50}$) |
|---|---|
| Hormone not added | 137 |
| -18 | 18 |
| 0 | 20 |
| + 5 | 37 |
| +10 | 152 |

Note: – before introduction; + after introduction of interferonogen.

interferon-inhibiting activity of the hormone. Analysis of the results in Table 1 shows a clear difference between the character of action of DLNDH on total cell protein synthesis and on interferon synthesis: in the first case the action of the hormone was reversible, but in the second it was irreversible.

The object of the next series of experiments was to determine the phase of interferonogenesis which is sensitive to the action of DLNDH. For this purpose the hormone was added to the growth medium at various times before or after infection with the interferonogen. In all experiments the interferon titer was determined 24 h after introduction of the interferonogen. The results of these experiments, given in Table 2, indicate that addition of the hormone 18 h before infection of the cells, simultaneously with infection, or 5 h thereafter led to marked inhibition of interferon production. Addition of the compound 10 h after infection had no effect on interferon production. The phase of interferonogenesis sensitive to the action of DLNDH was thus more than 5 h, but less than 10 h after infection of the cells. These findings are in agreement with the statement of Reinicke [5] that the phase of interferonogenesis sensitive to the action of some steroid hormones occurs for several hours after introduction of the virus. In the system used, interferon first began to be detected in the culture fluid 5 h after infection of the cells, and it was secreted into the medium on a large scale 10 h after infection. The action of DLNDH was thus observed in the initial phase of interferonogenesis, i.e., in the phase during which interferon production is inhibited by inhibitors of RNA synthesis [2, 3, 6, 7].

In the present experiments, interferon synthesis was also inhibited by actinomycin, olivomycin, and rubomycin C during the first few hours after introduction of the interferonogen.

It can be postulated on the basis of these results that the mechanism of action of DLNDH is associated with its effect on RNA synthesis taking place during interferonogenesis.

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